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# Biliary Structures Lead to Tumour Recurrences After Laser-Induced Interstitial Thermotherapy

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***Background and Objective:*** **Thermal diffusion during laser-induced interstitial thermotherapy (LITT) has not yet been fully investigated in heterogeneous tissue architecture such as liver. LITT was performed on rabbit liver tumours to analyse the role of biliary structures in thermal diffusion.**
***Study Design/Materials and Methods:*** **Twenty-four VX2 tumours were grafted onto 12 rabbit livers. The animals were randomly separated into two groups when tumour size reached 8 mm. Thermotherapy was performed by delivering the 830-nm output of a diode laser to the centre of the tumour with a 300-μm fibre. Irradiation conditions were 1.5 W over 900 sec. On day 7 or 14, the tumours were removed and stained with haematoxylin–eosin and picrosirius red F3BA (PR). Thermal damage was evaluated by PR and electron microscopic examinations.**
***Results:*** **Among the treated tumours, recurrences were found both at the periphery (one on day 7, seven on day 14) and within the treated area (two on day 7, two on day 14). All recurrences were located in the vicinity of the biliary structures, which are frequently spared from thermal injury.**
***Conclusion:*** **Biliary ducts lead to a heat sink, thereby facilitating tumour recurrences. Lasers Surg. Med. 24:269–275, 1999.**


**Key words: laser-induced interstitial thermotherapy; diode laser; liver tumours; thermoresistance; biliary structures**

## INTRODUCTION

The efficacy of therapies for hepatocellular carcinoma is poor [1,2]. The development of these tumours on cirrhotic tissue usually contraindicates extensive surgical procedures [3]. Nevertheless, a mini-invasive and percutaneous procedure that destroys the tumour and spares the hepatic tissue, such as percutaneous alcohol injection, is of therapeutic interest [4,5]. Percutaneous alcohol injection is indicated mainly for patients with uninodular tumours of at least 3 cm [4]. Because of the inhomogeneous diffusion of alcohol, the extent of necrosis is difficult to predict, in particular on the surrounding noncancerous liver parenchyma [5]. Moreover, treatment modalities (administration dose and treatment frequency) have not been well defined.

Laser-induced interstitial thermotherapy (LITT) has been proposed for the treatment of nonresectable hepatic tumours [6,7]. Coagulation necrosis induced by LITT has been reported to be predictable, without affecting adjacent tissues

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**TABLE 1. Comparison of Groups A and B***

| Groups | Weight of rabbit (kg) | Tumour diameter before treatment (mm) | Weight (kg) at death |
|---|---|---|---|
| A | | | |
| Average | 2.8 | 8.8 | 2.7 |
| SD | 0.5 | 1.7 | 0.5 |
| Extreme values | 2–3.1 | 7–11 | 2–3 |
| B | | | |
| Average | 3 | 10.2 | 2.9 |
| SD | 0.2 | 2.2 | 0.2 |
| Extreme values | 2.7–3.2 | 8–12 | 2.6–3 |
| Comparison | NS | NS | NS |

*Kruskal-Wallis test; NS, nonsignificant.

[6,7]. Diode lasers have been recently proposed for LITT [8,9]. Because of the relatively good penetration of near-infrared radiation, a deep tissue effect can be achieved. The small size of these lasers is also well adapted to the medical environment.

In the present study, the thermotherapeutic outcome of treating heterogeneous tissue architecture, such as that found in liver, is addressed. For this model, diode laser thermotherapy was performed on rabbit liver tumour (VX2 model) and histologically analysed.

## MATERIALS AND METHODS

### Materials

Twelve New Zealand 12-week-old rabbits (2.8 ± 0.3 kg) were anaesthetised by intramuscular injection of ketamine (40 mg/kg) and acepromazine (4 mg/kg). Twenty-four VX2 tumours were grafted onto 12 rabbit livers. The tumours were implanted separately in the left and the right lobes by median laparotomy.

The VX2 carcinoma was an undifferentiated tumour (ATCC designation: CRL 6503). All tumoural grafts, 2 mm in diameter, were obtained from the same initial tumour. The grafts were progressively frozen (Minicool®, DMC, Sassenage, France) and stored before implantation. Tumoural growth was controlled by ultrasound examinations (USL 500) with a 7.5-mHz sector transducer every three days. This method allowed us to obtain homogeneous tumour groups before treatment.

We used a custom-designed diode laser system developed at the Ecole Polytechnique Fédérale de Lausanne (Switzerland). The system produced a 830-nm continuous-wave laser output at the tip of a 300-μm optical fibre. The distal part of the bare fibre tip was implanted directly into the centre of the tumour.

### Methods

The animals were separated randomly into two groups (A and B) when the tumour reached an average size of 8 mm in diameter. Tumours of each group were treated by LITT with the same laser parameters (1.5 W over 900 sec, energy = 1,350 J, irradiance = 2,140 W/cm$^2$). Ten rabbits were included in the study. Two rabbits were excluded because there was no tumour development in one case and there was confluence of the two grafts in the other. Before treatment, no significant differences in rabbits and tumours were found in either group. Averages, standard deviations, and extreme values are presented in Table 1.

Livers were removed on day 7 for group A and on day 14 for group B. The irradiated areas were fixed in formalin and stained with haematoxylin–eosin (HE) and picrosirius red F3BA (PR). PR demonstrates the birefringence of normal collagen under polarized light; areas of denatured collagen have no optical activity. Because collagen denaturation occurs above 65°C, PR stains tissue heated above this temperature and allows the extent of thermal damage on examined slices to be defined [10].

For electron microscopic examination (Hitachi 7100 S microscope), strips of tissue were removed and divided into six parts, from the centre to the periphery of the irradiated area. The pieces, fixed in glutaraldehyde and osmic acid, were contrasted with uranyl acetate and lead citrate.

A screening for tumour recurrence was performed in groups A and B by microscopic examination. PR staining was used as the thermal damage indicator.

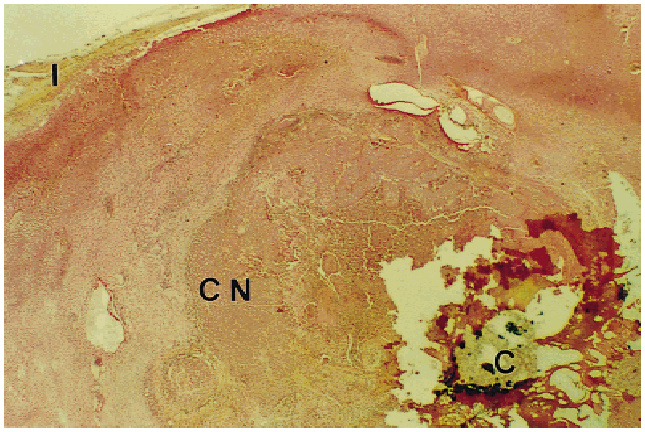


Fig. 1. A totally destroyed tumour with charring (C) and vacuolisation at the irradiated centre, coagulation necrosis (CN) in the surrounding area, and a peripheral inflammatory reaction (I). Haematoxylin–eosin, ×10.

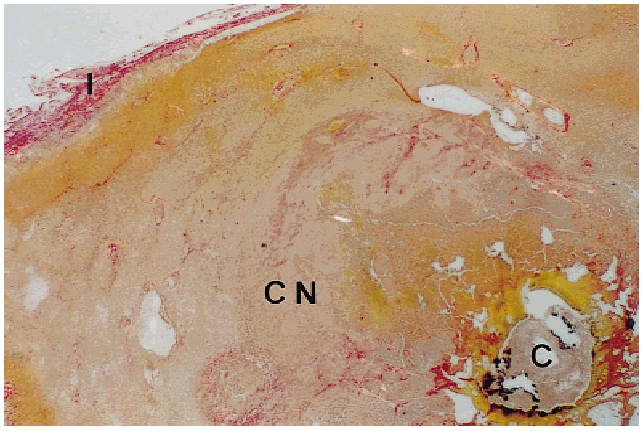


Fig. 2. The same tumour as that shown in Figure 1 was examined under polarized light with picrosirius red F3BA stain. Charring (C) and coagulation necrosis (CN) showed no optical activity, whereas the inflammatory reaction (I) showed a peripheral and well limited birefringence. ×10.

The quantitative data were compared with the Kruskal-Wallis test. Statistical significance was set at $P < 0.05$.

## RESULTS

Among the treated tumours, seven were completely destroyed at day 7 and only one at day 14.

In totally destroyed tumours, microscopic examination of the irradiated site after HE staining showed a circular and well-defined area (Fig. 1). The centre of the tumour was occupied by cavitation and charring, the surrounding zone was occupied by coagulation, and necrosis consisted mostly of destroyed tumour and necrotic hepatic tissue. The nuclei were pycnotic, and cytoplasmic membranes were not well individualized. Surrounding the site, an important and constant inflammatory reaction precisely defined the irradiated area (Fig. 1). On day 7, at the periphery of the irradiated area, intact biliary ducts were found scattered within the coagulation necrosis. After PR staining, coagulation necrosis showed no optical activity under polarized light, whereas the inflammatory reaction showed a peripheral, well-demarcated, and circular birefringence (Fig. 2). In contrast, the normal hepatic tissue, which is poor in collagen, showed very low birefringence. Electron microscopic examinations showed coagulation necrosis in the irradiated area where tumoural cells were totally destroyed. The nuclear and cytoplasmic membranes had completely disappeared, as did the cellular elements. At the periphery of the necrosis area, biliary ducts appeared to have been spared and were surrounded by dramatically altered tumoural cells (Fig. 3). Inflammatory cells, which distinguished the irradiated area from normal hepatocytes, were well individualized and intertwined by collagen.

In recurrences, because of the collagen-rich tumour stroma, anarchic birefringence was demonstrated with PR staining. Most recurrences were located at the periphery of the irradiated area, i.e., away from the inflammatory ring (Fig. 4). They were more frequent at day 14 (seven recurrences vs. one recurrence). Striking recurrences were found inside the irradiated area in both groups, all located near the biliary ducts (Fig. 5). The mean distance of these recurrences from the irradiation centre was 3.8 (SD = 1.5) mm, clearly inside the thermal diffusion zone, which was found to have a mean extension of 11.9 (SD = 2.6) mm on the fixed specimen. The recurrence distribution determined by HE and PR on day 7 (group A) and on day 14 (group B) is presented in Table 2. A good correlation was found between the different stainings used (HES and PR) to determine the recurrences (correlation coefficient, $\kappa = 0.83$; $P < 0.001$).

The pretherapeutic size of the completely destroyed and recurrent tumours did not differ significantly (mean ± SD: 9.8 mm ± 1.8 vs. 9.3 mm ± 2.3).

## DISCUSSION

The present study shows that the biliary duct has an influence on the recurrence of an ag-

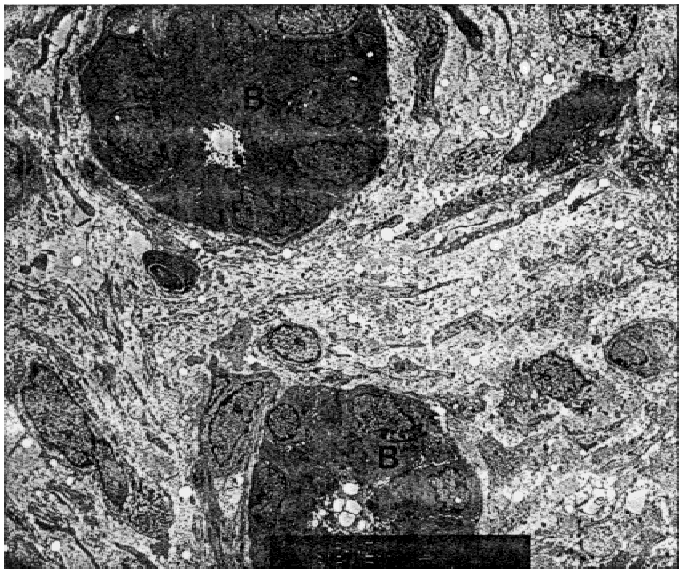


Fig. 3. Intact biliary ducts (B) at the periphery of the coagulation necrosis area. Electron microscopy, ×1,500.

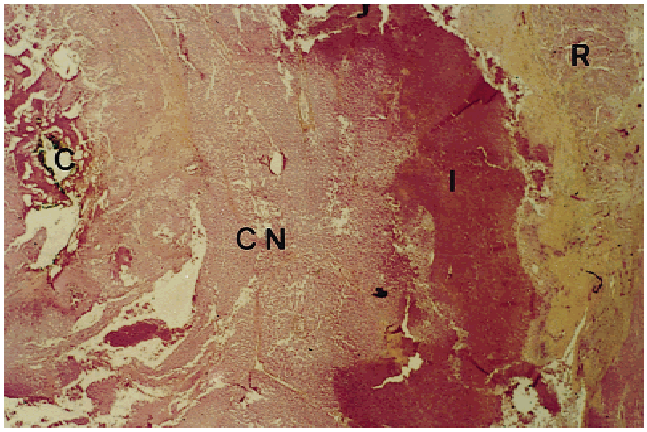


Fig. 4. Peripheral recurrence (R) located behind the inflamed ring (I). C, charring; CN, coagulation necrosis. Haematoxylin–eosin, ×10.

gressive hepatic tumour treated by LITT. The treatment of VX2 carcinoma experimentally induced in rabbit liver was apparently effective on day 7, but the results changed rapidly by the recurrences that appeared during the short-term follow-up.

The present study demonstrates that two types of recurrence must be distinguished: infrequent central ones located in the core of the tumour and appearing on either day 7 or 14, and more frequently peripheral ones located away from the inflamed ring, i.e., between the normal hepatic parenchyma and the irradiated tumoural tissue. These peripheral recurrences appeared more frequently on day 14.

The peripheral and latest recurrences can be explained by the insufficient destruction of peritumoural hepatic tissue. On day 7, the presence of intact biliary ducts at the periphery of the necrosis area indicated insufficient heating and explains the obvious peripheral recurrences on day 14 (Figs. 2, 4). Our previous study on subcutaneous tumours in mice indicated this "peripheral" limitation of LITT in a relative homogeneous tissue [9]. To avoid peripheral recurrences, a safety margin of 10 mm of hepatic tissue surrounding the tumour must be also destroyed by LITT, as has been reported for hepatic surgery [11]. The peritumoural tissue is the limitation zone of the LITT, where thermal destruction is uncertain, and thus facilitates peripheral tumoural recurrences. Simultaneous heating with multiple fibres or several irradiations with a single fibre may optimise results with LITT [12,13]. Nevertheless, placing fibres remains difficult to organize and the results are not easily predictable. The use of diffusors at the fibre tip is still being debated. For some researchers [7,14], the use of diffusors are not advantageous because of charring and fragments of tissue encumbering the

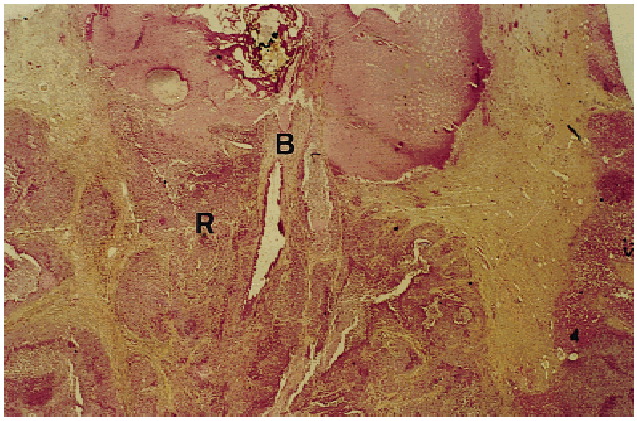


Fig. 5. Central recurrences (R) located just behind a biliary duct (B). Haematoxylin–eosin, ×10.

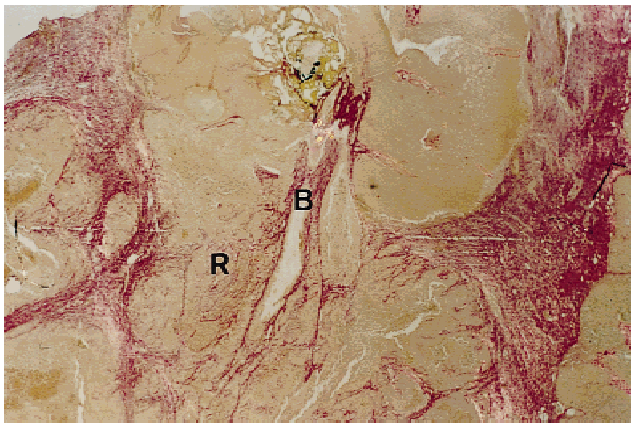


Fig. 6. The same tumour examined in polarized light using picrosirius red F3BA stain. Note the persistent birefringence of the biliary duct (B) and the recurrence (R) located just behind the duct. ×10.

**TABLE 2. Tumoral recurrences in the Centre and the Periphery of the Irratiated Site on Days 7 (Group A) and 14 (Group B); Correlation Between HE and PR Stains**

| Stain[a] | Recurrences | Group A (n = 10) | Group B (n = 10) |
|---|---|---|---|
| HE | Center | 2 | 2 |
| | Periphery | 1 | 7 |
| PR | Center | 2 | 1 |
| | Periphery | 1 | 7 |

[a]HE, haematoxylin–eosin; PR, picrosirius red F3BA.

fibre tip. In the studies by Malone et al. and Shi and Dachan [14,15] who compared three types of fibre tips and diffusors, the simple bare tip produced the largest diameter of necrosis. Amin et al. [7] recommended precharring the tip to increase the size of necrosis.

Peripheral recurrences, especially when thermal destruction is incomplete in this area, are also facilitated by the aggressive cancerous properties of our tumour model. The VX2 carcinoma is an undifferentiated and infiltrating tumour as opposed to hepatic tumours such as hepatocarcinoma, which are usually well demarcated and even encapsulated.

Striking central recurrences were found close to the fibre tip. They were all located behind a biliary duct. Thus, insufficient treatment was associated with the biliary ducts (Figs. 5, 6). This resistance was correlated with a reduction of the thermal diffusion as confirmed by the PR stain (Fig. 6). When using this "thermal" stain, tissues located just behind the ducts were still birefringent, indicating a thermal decrease. Similarly, intact biliary ducts were found at the periphery of the coagulation necrosis, indicating insufficient heating in the area of the biliary ducts, which explains the heat sink and the central recurrences. Several hypotheses can be proposed to explain the resistance of biliary ducts to thermal effects: (a) the endodermal structure of the ducts may be resistant to heat, (b) the structure of the portal space where the biliary ducts are located is cooled by a portal vein and a hepatic artery, and

(c) the tubular structure of the biliary ducts may have a screen effect that alters the diffusion of heat behind the duct. This effect would protect tissue adjacent to the biliary duct.

If hepatic vascularisation contributes to the decrease in heat, clamping the hepatic pedicle could limit the vascular cooling effect, but this procedure would indicate a laparotomy.

In the present study, PR was coupled with HE to indentify recurrences. With regard to the determination of recurrences, the two staining techniques were well correlated (Table 2). PR greatly enhances the natural birefringence of normal collagen fibres under polarised light [10], whereas thermically denatured collagen heated to 65°C does not show birefringence. In contrast to hepatic tissue, tumour stroma is rich in collagen; these histologic properties allow a good differentiation of tumoural tissue from normal tissue. PR is a specific technique to evaluate the results and limits of a thermal therapy on histologic sections.

In situ thermal dosimetry has to be developed to detect insufficient thermal distribution. Magntic resonance imaging (MRI) using thermosensitive sequences is being used to investigate the thermal damage induced by LITT [16–18]. Real-time MRI dosimetry should control the treatment of hepatic tumours with LITT. To avoid central recurrences, biliary ducts and the hepatic vasculature should be localised in the irradiated area. Great care should be taken during the treatment of tissue modifications surrounding these structures.

Undestroyed tumoural tissue should be clearly distinguished from normal liver and necrotic tissues. In case of insufficient treatment, the LITT procedure should be repeated and the fibre should cover the untreated area located behind biliary ducts or hepatic vessels.

This MRI-adapted treatment would be more efficient than simply changing laser parameters (time or power). To minimise the peripheral recurrences and destroy tumours, a 1-cm safety margin of peritumoural tissue must also be identified and destroyed. Further studies are in progress to evaluate the use of real-time MRI to control the quality and efficiency of LITT on hepatic tumours.

## CONCLUSION

Peripheral and striking central recurrences after LITT are the consequence of a heat sink induced by the biliary ducts and the hepatic vasculature. Dosimetry by MRI thermosensitive sequences should allow total destruction of the tumours.

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